Modulation of Radiation – Induced Tumor Necrosis Factor α (TNF- α) Gene Expression in the Rats Spinal Cord by Melatonin

G.H. Haddadi[1], A. Shirazi[2], Z. Sepehrizadeh[3] and M. Haddadi[4], S.Refahee[2]

[1] Medical Physics Department, Fasa University of Medical Sciences, Fasa , Iran
[2] Medical Physics Department, Tehran University of Medical Sciences, Tehran, Iran
[3]Depatment of Biothecnology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[4] Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract— **The spinal cord is the major dose- limiting organ for radiotherapy of cancer in the head and neck region. Understanding the cellular and molecular mechanisms may help to develop strategies to either increase the radiation tolerance or to treat spinal cord alterations induced by irradiation. Radiation exposure leads to oxidative stress and necrosis in many cell types including neurons. Radiation – induced apoptosis has also been observed in animal models. The exact mechanism and the genes that are activated in the process of radiation- induced apoptosis has not established yet. However many events that occur at the cell surface and intracellular during apoptosis in the nervous system have been reported. There is literature to support possible roles for TNF- α as a contributor to apoptosis in the CNS. In the last decade, there have been reports on the anti apoptotic effect of melatonin, an endogenous compound mainly synthesized by the pineal gland in the human brain. The purpose of this study was to investigate changes in TNF- α gene expression in the spinal cord after neck irradiation with 22 Gy. In addition, we evaluated the ability of melatonin to modulate the radiation - induced TNF- α gene expression in this animal model of spinal cord irradiation. A number of rats were divided into four groups: 1. Control group; 2. Group that was treated with intraperitonial injection of melatonin; 3.Group of rats which got melatonin 30 min. prior to cervical spinal cord gamma irradiation at a dose of 22 Gy; and 4.Group that was given an intraperitonial injection of vehicle and the spinal cord radiation. The expression of TNF- α was evaluated by real time reverse transcription polymerase chain reaction (Real time RT-PCR). The finding indicates that melatonin down-regulates the TNF- α gene expression in the spinal cord in response to radiation.**

Keywords— **Spinal cord, Radiotherapy, TNF- α, Melatonin**

INTRODUCTION

Most patients who are currently diagnosed with head and neck malignancy will receive a course of radiotherapy for cure or for local control or symptomatic palliation.[1] The potential consequences of damaging healthy spinal cord within the radiation field can be severe and limit the dose of radiation which can be delivered to tumors in this region. Almost 40% of patients who received cumulative radiation doses of 60 Gy showed histological evidence of radio necrosis at the time of rebiopsy.[2] Severity of clinical symptoms of radiation myelopathy depends on the level and the affected region of spinal cord and it may be not diagnosed from the associated symptoms alone. The underlying mechanisms of this injury remain unclear, however, there is an increasing body of data indicating that the response of the CNS after irradiation is a continuous, dynamic, and interacting process.[3] TNF-α has already been introduced as a potential trigger for apoptosis. However, depending on the context, this protein appears capable of exerting opposite effects on oligodendrocyte apoptosis. Most studies have demonstrated primarily toxic effects, leading to apoptosis in several different models. In addition, TNF- α exerts in particular pro inflammatory effects by inducing the expression of adhesion molecules that recruit leukocytes into the sites of tissue damage, by priming leukocytes for oxidant production, and by inducing production of prostaglandins and other mediators of inflammation. Also, TNF- α exerts fibrogenic effects by stimulating the growth of fibroblasts and increasing the collagen deposition.[4] Therefore, a pharmacological regulation of the TNF- α production at the initial stage could possibly modulate the progression of radiation-induced injury. In the last decade, there have been reports on the radioprotective effect of melatonin, an endogenous compound mainly synthesized by the pineal gland in the human brain. Melatonin (Nacetyl-5-methoxytryptamine) is a ubiquitously acting molecule with several functions. It is highly lypophilic and somewhat water-soluble. The widespread cellular distribution of melatonin may allow it to interact with all molecules, thereby reducing the oxidative damage to molecules in both lipid and aqueous environments of the cell. It has been reported that melatonin directly scavenges the highly toxic hydroxyl radicals both in vitro and in vivo, as well as several other reactive species such as single oxygen, peroxynitrite anion.[5] Its free radical–scavenging capacity is mediated by electron donation. Several studies have shown that melatonin reduces chronic and acute inflammation.[6,7,8,9] The immunomodulatory and antiapoptotic properties of melatonin are well known; it acts on the immune system by regulating cytokine production of immunocompetent cells.[10] Experimental and clinical data showing that melatonin reduces adhesion molecules and pro-inflammatory cytokines and modifies serum inflamma-

M. Long (Ed.): World Congress on Medical Physics and Biomedical Engineering, IFMBE Proceedings 39, pp. 38–40, 2012.
www.springerlink.com

tory parameters. After SCI, TNF- α might serve as an external signal, initiating apoptosis in neurons and oligodendrocytes. The inhibition of the MMP-2, and MMP-9 by melatonin is most likely attributed to the suppressive effect on TNF- α production.[11] The results of different studies indicate that both the acute and the chronic toxicities of melatonin are extremely low.

I. MATERIAL AND METHOD

The rats were divided into four groups. The first group (vehicle treatment) served as control. The second group (radiation) was treated with vehicle and 30 min later, the rats were exposed to radiation, which will be detailed in the following section. The third group (radiation+melatonin) was given an intraperitonial injection of melatonin (100 mg/kg body weight) and 30 min later exposed to radiation in the same manner as in the second group. The fourth group (melatonin-only) was also given an intraperitonial injection of melatonin (100 mg/kg body weight).

Irradiation

The animals were anesthetized with an i.p injection of ketamin (60 mg/kg) and xylazin (20 mg/kg). Then, they were placed in a prone position. The rats of groups 2 and 3 were irradiated with the gamma beam of Cobalt-60 teletherapy unit (theratron 760-C) to the 1.8 cm cervical segment of the spinal cord (C1-T2). A single dose of 22 Gy at the dose rate of 1.8 Gy/min and source skin distance of 79.5cm was administered to the depth of 0.5 cm based on lateral simulation radiographs. This dose is proposed as the "Effective Dose" for white matter necrosis and limb paralysis after 20 weeks of irradiation.[12] Sham irradiation was also performed for control and melatonin-only groups. The rats were anesthetized, but were not irradiated. The animals were scarified under ketamine and xylazine injection chronologically in 4 and 24 hours, and 1, 3, 8, 16, 20, and 22 weeks after radiation therapy. Five rats were used at each time point. Tissue sampling was done through posterior approach to cervical spinal cord. One centimeter of spinal cord was dissected and used for real time RT-PCR study. To this purpose, the spinal cord was embedded in GITC (6 molar) for the inactivation of enzymes and RNase-free conditions, and then homogenized by Heidolf Homogenizer. All samples were stored at -70^{o} C until required.

The PCRs were carried out in a reaction volume of 25 μL consisting of 5 μL cDNA, 20 pmol of each primer, and 12.5 μL PCR-mix. Thermal cycling was initiated with an initial denaturation step of 94 °C for 3 min and followed by the thermal profile of 94 °C (20 s) + 55 °C (30 s) + 72 °C (40 s) for 40 cycles in a Stratagen real-time system. A suitable threshold was applied to amplification plots, and the resulting C_t values (threshold cycles) were used for relative quantification. The Ct values of VEGF were normalized according to the Ct values of β-actin, and the resulting values were compared with those of the control group using a $2^{-\Delta\Delta ct}$ method.

II. RESULTS

After a single dose of 22 Gy, TNF- α gene expression was measured in the experimental groups, it was defined proportionate with the age-matched controls after 4 and 24 hours and 1, 3 , 8, 16, 20 and 22 weeks after irradiation (fig. 1). At the early time after irradiation, Radiation did not induce elevations in TNF- α gene expression in the irradiated group.

Within 3 weeks after irradiation, TNF- α gene upregulated 3 fold in the irradiated group compared with the control group($p<0.05$). TNF- α expression in the irradiated group decreased over time as the interval after radiation is extended to 22 weeks. Within 16 and 20 weeks after irradiation, the expression of VEGF gene in the irradiated group was same as the control group. TNF- α expression in melatonin pretreatment group compared with the radiation group is significantly down-regulated at the 3 weeks after irradiation ($p<0.05$). There is not any difference between the control and melatonin-only groups.

III. CONCLUSIONS

TNF- α is released in the beginning of the immune response to endotoxin, physical injury and a variety of inflammatory processes. Up to now two major apoptosis mediating cascades have been analyzed in greater detail. The first pathway activated by radiation and cellular stress. Radiation induced apoptosis primarily relies on mitochondrial damage followed by caspase activation. Bax can induce release of pro-apoptotic molecules including cytochrome c and apoptosis inducing factor (AIF). The release of cytochrome c triggers the activation of caspase-9 via APAF-1 and dATP. The second pathway of apoptosis is mediated by members of the death ligand family including TNF- α, CD95-l, and TRAIL. After activation of a death receptor (i.e. TNFά-R1) the adapter FADD mediates activation of caspase-8. Adhering leukocytes may increase the vessel toxicity via TNF- α induced apoptosis.[4] TNF- ά has been shown to induce apoptosis in oligodendrocytes both in vitro and in vivo. Melatonin as a neuroprotector has used in some model of CNS injury. Neuroprotective effect of melatonin in Spinal cord injuries due to trauma, hypoxia, ischemia, and toxic agent have been shown by other researchers in the short time after injury.[10,13,14] It has been postulated that

in addition to scavenging the free radicals, probably melatonin can preserve mitochondrial homeostasis, and produce vasoconstriction in CNS arterioles. The exact mechanism of melatonin mediated to modulate TNF- α gene expression unclear. Probably, the regulation of TNF- α is complicated and in addition to the antioxidative effect, other transcriptional factors may be involved in the regulation of TNF- α gene expression by melatonin. Reiter noted that "melatonin does not function exclusively as a free radical scavenger and antioxidant, but may have other functions which help cells and organisms to cope with metabolic disasters. For example, melatonin can influence NF*k*B, a multifunctional transcription factor that is capable of influencing a variety of genes.[14] The precise role of melatonin in TNF- α down-regulation and neuroprotection after irradiation remain to be determined.

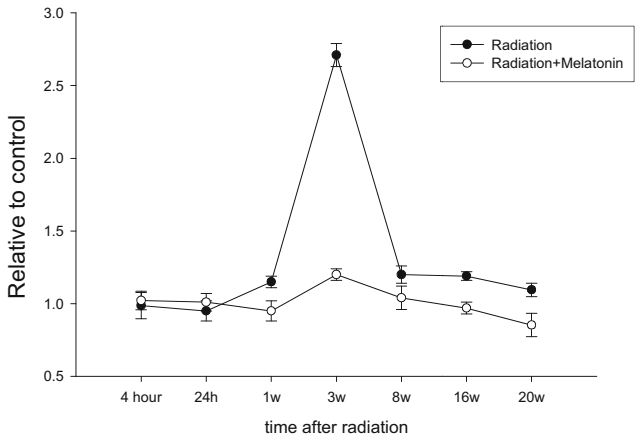

Fig. 1 **Changes in TNF-α gene expression after a single dose of 22 Gy and melatonin treatment. Each value represents the relative of the age-matched control animal at that time point. Vertical bars represent standard error of the mean.**

ACKNOWLEDGMENT

We are grateful to the staff of the Radiotherapy Department at Cancer Institute of imam khomaini hospital especially Mr. Amir Hooshang Sedigh for the great help.

REFERENCES

1. Wong C. Van der Kogel A (2004) Mechanisms of radiation injury to the central nervous system. molecular interventions **4**(5): 273-284
2. Bijl HP, Van luijik P, Coppes R P. (2003) Unexpected changes of rat cervical spinal cord tolerance caused by inhomogenous dose distributions. Int J Radiat Oncol Biol Phys 57**:** 274-281
3. Okada S, Okeda R (2001) Pathology of radiation myelopathy. Neuropathology. **21**: 274-256
4. Belka C, Budach W, et al. (2001) Radiation induced CNS toxicity – molecular and cellular mechanisms. British Journal of Cancer 85 (9):1233-1239
5. Shirazi A, Ghobadi G, Ghazi-Khansari M. (2007) A radiobiological review on melatonin: A novel radioprotector. J. Radiation. Res**.** 263-272
6. Shirazi A, Haddadi G H, Ghazi- Khansari M, Abolhassani F. Mahdavi S R. (2009) Evaluation of Melatonin for Prevention of Radiation Myelopathy in Irradiated Cervical Spinal Cord. Yakhteh. Medical Journal 11(1): 43-48
7. Serin M, Gulbas H, Gures I, Sukru H, Yucel N. (2007). The histopathological evaluation of the effectiveness of melatonin as a protectant against acute lung injury induced by radiation therapy in a rat model. Int. J. Radiat. Biol 83**:** 187-193
8. Shirazi A, Haddadi G H, et al.(2011) Radioprotective Effect of Melatonin in Reducing Oxidative Stress in Rat Lenses. Yakhteh. Medical Journal 13(2): 79-82
9. IFMBE Proc. vol. 25, World Congress on Med. Phys. & Biomed. Eng., Munich, Germany, 2009, pp 540–543
10. Shirazi A, Haddadi G H, B Minaee, et.al. (2010) Evaluation of melatonin for modulation of apoptosis-related genes in irradiated cervical spinal cord. Int. J. Low Radiation, Vol. 7, No. 6,436-445
11. Jennifer L Daigle, Ji-Hong Hong, et al.(2001) The role of TNF signaling pathways in the response of murine brain to irradiation. Cancer research61: 8859-8865
12. Nordal RA, Nagy A, Pintillie M, Wong CH. (2004) Hypoxia and hypoxia-inducible factor-1 target genes in central nervous system radiation injury: A role for vascular endothelial growth factor. Clinical Cancer Research;10:3342-3353
13. IFMBE Proc. vol. 14, World Congress on Med. Phys. & Biomed. Eng., Seoul, Korea 2006, pp 2192–2195
14. Reiter RJ (1998) Oxidative damage in the central nervous system: Protection by melatonin. Progress in Neurobiology359-384

Use macro [author address] to enter the address of the corresponding author:

Author: Gholamhassan Haddadi
Institute: Fasa University of Medical Sciences
Street: Avesina
City: Fasa
Country: Iran
Email: ghadadi@gmail.com